AMENDMENT 37 C.F.R. 1.111 Address to: Assistant Commissioner for Patents Washington, D.C. 20231	Attorney Docket	SEEK-003
	First Named Inventor	R. Ehrhardt
	Application Number	09/852,448
	Filing Date	May 9, 2001
	Group Art Unit	1633
	Examiner Name	Jon Angell
	Title: *Models of Chronic and Acute Inflammatory Disease*	

DECLARATION UNDER 37 C.F.R. §1.132

Sir:

I, Dr. Holger Karsunky, do hereby declare as follows:

I am scientist at Cellerant Therapeutics, Inc., a licensee of the present paten application. I have worked in the field of hematopoietic stem and progenitor cells for 11 years. A copy of my CV is attached. I have read and understood the Office Action of April 26, 2007, and the references cited therein, particularly with respect to the rejection of claims 1, 4, 7 and 8 as being unpatentable over Clay *et al.* (2001) Blood 97:1982.

The present claims are directed to a substantially pure composition of monopotent mammalian megakaryocyte progenitor cells, wherein at least 80% of the cells in said composition express CD41, CD9 and CD34 and do not express CD2; CD3; CD4; CD7; CD8; CD10; CD11b; CD14; CD19; CD20; CD56; and glycophorin A (GPA), and wherein the cells in said composition that express CD41, CD9 and CD34 and do not express CD2; CD3; CD4; CD7; CD8; CD10; CD11b; CD14; CD19; CD20; CD56; and glycophorin A (GPA) give rise exclusively to megakaryocytes and platelets. That a cell population with these characteristics would possess substantial progenitor potential and give rise exclusively to megakaryocytes and platelets would not have been expected based on the teachings of the prior art.

The paper by Clay *et al.* describes a population of $CD34^{+}CD9^{+}CD41^{-}$ bone marrow cells and provides data showing that this population has megakaryocyte progenitor potential but in addition posses' myeloid and erythroid potential. (see Clay et al., page 1985, column 1, last paragraph).

In the same paper the authors also investigate the potential of $CD34^+CD9^+CD41^+$ cells and state that in contrast to the $CD41^-$ fraction, $CD41^+$ cells "only gave rise to a small number of differentiated megakaryocytic clusters". No plating efficiency or number of colonies is given suggesting that the authors did not see any. The authors use of the word 'cluster' (instead of 'colonies' or 'CFU' as was stated for the readout from $CD41^-$ cells) is indicative that these group of cells were just stuck or attached together and were probably immediate precursors that differentiated into mature megakaryocytes without any proliferation. A true progenitor should have the capability to proliferate to a certain degree and form a colony of daughter cells from a single cell, which is the definition of a colony forming unit, CFU.

In stark contrast, NaNakorn *et al.* reports the generation of colonies from his $CD41^+$ megakaryocyte progenitor (MKP) at a high plating efficiency of up to 65% depending on the cytokine cocktail used (see Table 1& 2 as well as Figure 2C). These data unequivocally show that the $CD41^+$ fraction contains potent megakaryocyte progenitors at a high frequency that are able to proliferate and form a colony at the single cell level. The fact that only $3x10^4$ MKP cells produced substantial numbers of platelets *in vivo,* which is only possible if MKPs undergo substantial proliferation, further supports this finding.

In summary, the function attributed to the $CD34^+CD9^+CD41^+$ population by the two publications is clearly different and Clay *et al.* failed to identify the $CD41^+$ population as a potent megakaryocyte progenitor.

I hereby declare that all statements made herein of my own knowledge are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: 8/02/07

By _____

Holger Karsunky, Ph.D.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
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NAME	POSITION TITLE
Holger Karsunky	Senior Staff Scientist
eRA COMMONS USER NAME HolgerKarsunky	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Marburg, Germany	M.Sc.	1991-1996	Molecular Biology
University of Essen Medical School, Germany	Ph.D.	1996-2000	Immunology
University of Essen Medical School, Germany	Postdoctoral Fellow	2000-2001	early hematopoiesis and development of memory T cells
Stanford University, California	Postdoctoral Fellow	2001-2005	hematopoietic stem cells and progenitors

A. Professional Experience and Honors

2007 – current Senior Staff Scientist, Cellerant Therapeutics, San Carlos, CA
2005 – 2006 Research Staff Scientist, Cellerant Therapeutics, San Carlos, CA
2001-2005 Post-doctoral fellow, Stanford University School of Medicine, Department of Pathology (PI, Dr. Irving L. Weissman)
2000-2001 Post-doctoral fellow, University of Essen School of Medicine, Institute for Cell Biology and Cancer Research (PI, Dr. Tarik Möröy)
1996-2000 Graduate student, University of Essen School of Medicine, Institute for Cell Biology and Cancer Research (PI, Dr. Tarik Möröy)
1995-1996 Graduate student, University of Marburg (Dr. Tarik Möröy)

2001 – Postdoctoral Fellowship of the Ernst Schering Research Foundation
2000 – Earned doctoral degree with *summa cum laude* from the University of Essen
1996 – Doctoral Fellowship of the German Research Foundation (DFG)

B. Peer-reviewed Publications

1. Sanyal M, Tung JW, Karsunky H, Zeng H, Selleri L, Weissman IL, Herzenberg LA, and Cleary ML. B cell development fails in the absence of the Pbx1 proto-oncogene. Blood 109: 4191-4199, 2007.
2. Mende I, Karsunky H, Weissman IL, Engleman EG, and Merad M. Flk2^{+} myeloid progenitors are the main source of Langerhans cells during inflammatory conditions. Blood 107: 1383-1390, 2006.
3. Karsunky H, Merad M, Mende I, Manz MG, Engleman EG, and Weissman IL. Ontogeny of Interferon alpha Producing Dendritic Cells. Exp. Hematol. 33: 173-181, 2005.
4. So CW, Karsunky H, Wong P, Weissman IL, and Cleary M. Leukemic transformation of hematopoietic progenitors by MLL-GAS7 in the absence of Hoxa7 or Hoxa9. Blood 103: 3192-3199, 2004.
5. Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, and Weissman IL. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. Genes & Develop.17: 3029-3035, 2003.

6. Adhikary S, Peukert K, Karsunky H, Lutz W, Elsässer HP, Möröy T, and Eilers M. Miz1 is required for early embryonic development during gastrulation. Mol. Cell Biol. 23: 7648-7657, 2003

7. Karsunky H, Merad M, Cozzio A, Weissman IL, and Manz MG. Flt3 ligand regulates dendritic cell development from Flt3-positive lymphoid and myeloid committed progenitors to Flt3-positive dendritic cells in vivo. J. Exp. Med. 198: 305-313, 2003.

8. Yücel R*, Karsunky H*, Klein-Hitpass L, and Tarik Möröy. The transcriptional repressor Gfi1 affects development of early, uncommitted c-Kit+ T-cell progenitors and CD4/CD8 lineage decision in the thymus. J. Exp. Med. 197: 831-844, 2003.

9. So CW, Karsunky H, Passegue E, Cozzio A, Weissman IL, and Cleary ML. MLL-GAS7 transforms multipotent hematopoietic progenitors and induces mixed lineage leukemias in mice. Cancer Cell 3: 161-71, 2003.

10. Geisen C*, Karsunky H*, Yücel R, and Möröy T. T-cell lymphoma in CD2-cyclin E transgenic mice that are deficient for p27Kip1. Oncogene 22: 1724-1729, 2003.

11. Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, Weissman IL, Cyster JG, and Engleman EG. Langerhans cells renew in the skin throughout life under steady-state conditions. Nat. Immunol. 3:1135-41, 2002

12. Karsunky H , Zeng H, Schmidt T, Zevnik B, Kluge R, Schmid KW, Dührsen U, and Möröy T. Inflammatory reactions and severe neutropenia in mice lacking the transcriptional repressor Gfi1. Nature Genetics 30: 295-300, 2002.

13. Karsunky H, Mende I, Schmidt T, and Möröy T. High levels of the onco-protein Gfi-1 accelerate T-cell proliferation and inhibit activation induced T-cell death in Jurkat T-cells. Oncogene 21: 1571-1579, 2002.

14. Staller P, Peukert K, Kiermaier A, Seoane J, Lukas J, Karsunky H, Möröy T, Bartek J, Massague J, Häne F, and Eilers M, Repression of p15INK4b expression by Myc through association with Miz-1. Nature Cell Biol. 3: 392-399, 2001.

15. Rödel B, Tavassoli K, Karsunky H, Schmidt T, Schaper F, Heinrich P, Shuai K, Elsässer HP, and Möröy T. The zinc finger protein Gfi-1 can enhance STAT3 signaling by interacting with the STAT3 inhibitor PIAS3. EMBO J. 19: 5845-5855, 2000.

16. Beier R, Burgin A, Kiermaier A, Fero M, Karsunky H, Saffrich R, Möröy T, Ansorge W, Roberts J, and Eilers M. Induction of cyclin E-cdk2 kinase activity, E2F-dependent transcription and cell growth by Myc are genetically separable events. EMBO J. 19: 5813-5823, 2000.

17. Leduc I*, Karsunky H*, Mathieu N, Schmidt T, Verthuy C, Ferrier P, and Möröy T. The Pim-1 kinase stimulates maturation of TCRbeta-deficient T cell progenitors: implications for the mechanism of Pim-1 action. Int. Immunol. 12: 1389-96, 2000.

18. Schmidt T, Karsunky H, Fraß B, Denzel A, Baum W, and Möröy T. A novel protein –Fbf-1– that binds to CD95/Apo-1/Fas and shows sequence similarity to trichohyalin and plectin. Biochim. Biophys. Acta 91447: 1-6, 2000.

19. Möröy T, Karsunky H. Regulation of pre T-cell development. Cell. Mol. Life Sci. 57: 957-975, 2000.

20. Napirei M, Karsunky H, Zevnik B, Stephan H, Mannherz HG, and Möröy T. Systemic Lupus Erythematosus (SLE) in Dnase 1 deficient mice. Nature Genetics 25: 177-181, 2000.

21. Karsunky H, Geisen C, Schmidt T, Zevnik B, Gau E, and Möröy T. Oncogenic potential of cyclin E in Tcell lymphomagenesis in transgenic mice: Evidence for cooperation between cyclin E and Ras but not Myc. Oncogene 18: 7816-7825, 1999.

22. Schmidt T, Körner K, Karsunky H, Korsmeyer S, Müller R, and Möröy T. The murine Bax promoter is regulated by Sp1/3 and E-box binding proteins but not by p53. Cell Death Diff. 9: 873-882, 1999.

23. Schmidt T, Karsunky H, Zevnik B, Elsässer HP, and Möröy T. Zinc fingerprotein Gfi-1 has low oncogenic potential but cooperates strongly with Pim and Myc genes in T-cell lymphomagenesis. Oncogene 17: 2661-2668, 1998.

24. Schmidt T, Karsunky H, Rödel B, Zevnik B, Elsässer HP, and Möröy T. Evidence implicating Gfi-1 and Pim-1 in pre T-cell differentiation steps associated with beta-selection. EMBO J. 17: 5349-5359, 1998.

25. Haas K, Johannes C, Geisen C, Schmidt T, Karsunky H, Blass-Kampmann S, Obe G, and Möröy T. Malignant transformation by cyclin E and Ha-ras correlates with resistance against cell death but requires functional Myc and CDK4. Oncogene 15: 2615-2624 1997.

26. Zörnig M, Schmidt T, Karsunky H, Grzeschiczek A, and Möröy T. Zinc finger protein GFI-1 cooperates with myc and pim-1 in T-cell lymphomagenesis by reducing the requirements for IL-2. Oncogene 12: 1789-801, 1996.

C. Research Support.

Ongoing Research Support

2 R44 AI064156-02 Mandalam (PI) 06/15/06 - 06/14/08

Application of Expanded Myeloid Progenitors for Infection
Role: Co-PI

Completed Research Support

1 R43 AI064156-01 Karsunky (PI) 06/15/05 - 06/14/06

Application of Expanded Progenitors against Infection
Role: PI

1 R43 AI061856-01 Karsunky (PI) 07/01/2004 – 12/31/2006

Expansion of HSC for rescue in biodefense applications
Role: PI